Protocol for: Toxicokinetics of Ammonium Perchlorate (AP) and Iodine (I)

Submitted by: AFRL/HEST

Objective:

Contamination of ground water with ammonium perchlorate has raised important toxicological questions concerning the risk posed to potentially exposed human populations. Among several toxicological issues, two key kinetic factors will impact the risk analysis of AP:

- the dose of perchlorate at the thyroid gland under expected conditions of exposure, and

- the quantitative impact of perchlorate exposure on systemic kinetics of iodine.

The available literature is not sufficient to evaluate these factors with reasonable certainty. Without this information, several key issues can not be addressed and the final risk analysis will reflect the uncertainty in these factors. The proposed toxicokinetic studies will provide the database necessary to evaluate the impact of these kinetic factors on the perchlorate risk evaluation. Specific rat studies are proposed to investigate the overall systemic kinetics of perchlorate in order to evaluate both the bioavailability of perchlorate and the target tissue (thyroid) dosage under expected conditions of exposure. Within the context of these studies, a quantitative biologically based kinetic (BBK) model for perchlorate in rats will be developed and the model extrapolated to predict kinetics in humans. Additional studies are proposed to evaluate the impact of perchlorate on the kinetics of iodine (an essential element and a component of the thyroid hormone, thyroxine). These studies will provide necessary quantitative information on the dose-response relationship at a critical mechanistic step in the regulation of thyroid function.

Materials And Methods:

Experimental Design and General Procedures:

Toxicokinetics of Perchlorate

Standard *in vivo* toxicokinetic studies following single doses of sodium perchlorate, both i.v. and oral, will be conducted. Sodium perchlorate was chosen due to its high solubility in water, and the cation should not affect the kinetics of the perchlorate anion.

A preliminary study on the disappearance of perchlorate from blood and its tissue distribution will be conducted. Male Fischer 344 rats (n=3 for each sample time) will be dosed either via the tail vein (1 mmole/kg) or by gavage (3 mmole/kg). These doses are based upon literature data for an ip injection of perchlorate in male Sprague-Dawley rats

(Chow *et al.*, 1969, 1970) and are not expected to cause toxicological responses. Sodium perchlorate will be dissolved in 0.9% physiological saline and adjusted to pH of 7.4. Rats will receive an injection volume of 1 mL/kg. For the preliminary study, proposed sample points are 5 and 30 min and 2, 4, and 24 h post dosing. For the 24 h exposure, animals will be housed in metabolism cages for collection of urine and feces. Water and food will be available *ad libitum* during the experiments. Rats will be euthanized by CO_2 at the preset time point for i.v. and oral dosing. Blood, liver, kidney, thyroid, and muscle will be removed for analysis. Upon determination of hematocrit, blood will be centrifuged to separate plasma and red blood cells (RBCs). Protein in plasma, lysed RBCs, and urine will be precipitated with trichloroacetic acid (TCA) and the mixture will be centrifuged. Supernatant will be used to determine the concentration of perchlorate (Goldman *et al.*, 1973) according to an existing Standard Operating Procedure (SOP AC-96-36). Detection limit of this procedure is 0.005 μg/mL. Liver, kidney, thyroid, and muscle will be homogenized with water and will be treated with TCA to precipitate protein. After centrifugation the supernatant will be analyzed for perchlorate (SOP AC-96-36). For fecal analysis, a mixture of grounded feces and water will be vortexed for 24 h, centrifuged, and perchlorate anion determined in the supernatant (SOP AC-96-36).

Based on the results of the preliminary study, a full kinetic study will be designed. Three doses will be selected for both iv and oral exposures. Sampling time points will be selected to provide maximum information content of data collected. Groups of rats (n=4 for each sample time) will be exposed and data collected as described above. Comparison of the kinetic data for oral versus iv exposure will allow for the calculation of a bioavailability factor.

A recently developed biologically based kinetic (BBK) model for water soluble chemicals will be used to interpret the kinetic data and will form the basis for extrapolation from rats to humans.

Effect of perchlorate on iodine kinetics.

A dose-response relationship for the effect perchlorate on the systemic kinetics of iodine will be developed. Three groups of 8 rats will be exposed to ammonium perchlorate in drinking water for 14 days. The doses will be determined on the basis of the outcome of the 14 days toxicity studies currently being conducted. A group of 8 control rats will be maintained under identical conditions. At the end of exposure, rats will be dosed with $Na^{125}I$ (25μCi) by gavage to investigate the systemic distribution at 2 and 24 h. Blood, liver, kidney, thyroid, and muscle will be removed for analysis. Urine and feces will be collected and cumulative distribution determined. Iodine radioactivity in each tissue group will be counted by Packard γ-counter. No special sample preparation is required to count radioactivity. The effect of perchlorate treatment on the systemic distribution of iodide will be related to dose at the two time points. The early time point (2h) reflects uptake and distribution while the later time point (24h) provides information on the elimination phase. Since iodine delivery to the thyroid is a controlling factor in regulation of thyroid

function (Chow *et al.*, 1969; Schonbaum *et al.*, 1965) the data provided by these studies will allow for better mechanistic interpretation of functional effects observed.

References

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Chow SY and Woodbury DM. (1970), Kinetics of distribution of radioactive perchlorate in rat and guinea-pig thyroid glands. *J Endocr.* **47**, 207-218.

Goldman SJ and Stanbury JB. (1973). The metabolism of perchlorate in th rat. *Endocrinology* **92**, 1536-1538.

Schonbaum E, Sellers EA and Gill MJ. (1965). Some effects of perchlorate on the distribution of ^{131}I. *Acta Endocrinol.* **50**, 195-201.